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# Removal of benzo(*a*)pyrene in soil composting systems amended with the white rot fungus *Phanerochaete chrysosporium*

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**Abstract**

Inoculation of the benzo(*a*)pyrene (B(*a*)P) contaminated soil composting system with the white rot fungus *Phanerochaete chrysosporium* increased the rate of bound residue formation of contaminant carbon from 0.73 mg B(*a*)P/kg-day to 1.58 mg B(*a*)P/kg-day over the first 30 days of investigation. Despite this initial enhancement in contaminant removal, fungal inoculation was found to be ineffective in significantly enhancing the extent of benzo(*a*)pyrene during the 95 day composting study. The extent of contaminant removal was 62.8 ± 5.9%, 65.6 ± 1.2% and 49.3 ± 0.5% for the fungal inoculated, fungal uninoculated and poisoned compost reactors, respectively. First order modeling of removal kinetics yielded contaminant half lives of 11.5, 8.6 and 230 days for the fungal inoculated, uninoculated and poisoned compost reactor systems, respectively. Analysis of soil systems revealed appreciable numbers of PAH degrading fungi ($> 1 \times 10^4$ CFU/gm) in the uninoculated compost systems at the end of the 95 day treatment period.

Analysis of volatile traps indicated that neither mineralization nor vapor partitioning of benzo(*a*)pyrene (or its chemical intermediates) was significant during the soil composting process. Bound residue formation was found to be the predominant transformation mechanism for benzo(*a*)pyrene in the microbially active compost systems accounting for nearly 100% of the benzo(*a*)pyrene removed.

## 1. Introduction

Historically, the practice of land farming has been used to treat organic wastes generated in the petroleum and wood preserving (e.g., creosote) industries. Since promulgation of the 1984 Land Ban Legislation (Hazardous and Solid Waste Amendments of 1984 – HSWA), land farming of most of these wastes has ceased [1].

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However, because many of these wastes contained toxic compounds that degraded slowly in the natural environment, soils formerly used in industrial land farming operations may now pose a significant risk to public health and the environment [2, 3]. In addition to former land farming operations, accidental spillage and misguided disposal of petroleum and creosote wastes have resulted in extensive contamination of many soils by toxic organic compounds including polynuclear aromatic hydrocarbons (PAHs) [4].

PAHs are multiple ringed aromatic compounds which, as a group, account for nearly 85% of coal tar creosote [5]. Of the 16 PAH compounds found on the US Environmental Protection Agency's (EPA) priority pollutant list, it has been found that the higher molecular weight compounds (i.e., those containing four or more fused benzene rings) are resistant to biological transformation and persist in contaminated soil environments [6].

Recently, enclosed reactor systems were found to be effective in biologically treating soils contaminated with PAH compounds [7, 8]. The principal advantage of reactor systems over in situ treatment systems are better process control and selective enrichment of specific PAH degrading microbial communities. One of the most promising reactor systems for hazardous soil treatment is composting. Recent reports have shown that soil composting can be utilized to treat soils contaminated by a variety of hazardous organic compounds including PAHs, explosives and pesticides [7, 9, 10].

### *1.1. Soil composting*

Traditionally, composting has been characterized by an organically enriched thermophilic (50–55 °C) environment containing biodegradable organic matter, bulking agents, nutrients and moisture [11]. More recent applications of composting technology have broadened the definition to include process operation under psychrophilic (i.e., below 30 °C) conditions and operation with less than 50% organic amendment [7, 9].

Several microbial species that can degrade the higher molecular weight PAH compounds (e.g., pyrene, benzo(*a*)pyrene, etc.) have been identified [12–15]. However, only the white rot fungus, *Phanerochaete chrysosporium*, has been demonstrated to be effective in enhancing the removal of these compounds in hazardous soil composting systems [8].

The goal of the present study was to evaluate the effects of compost bioaugmentation using the white rot fungus *Phanerochaete chrysosporium* for treatment of PAH contaminated soil.

## 2. Materials and methods

Due to its known carcinogenic and mutagenic potential, benzo(*a*)pyrene (B(*a*)p) was chosen as the target PAH compound to be monitored during soil composting treatment. The chemical structure of benzo(*a*)pyrene is given in Fig. 1.

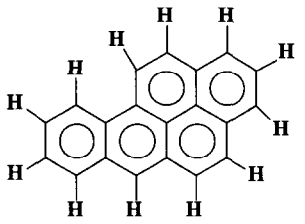

Fig. 1. Chemical structure of benzo(*a*)pyrene.

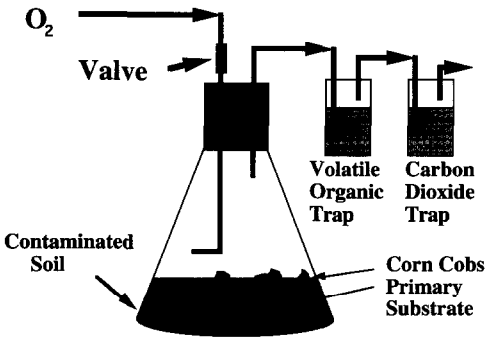


Fig. 2. Schematic of hazardous soil compost reactor.

The rate and extent of benzo(*a*)pyrene removal were estimated in three separate compost treatment systems that included, fungal amended, fungal unamended, and poisoned control systems. Each treatment system was monitored concurrently in order to quantify the effects of fungal bioaugmentation on PAH removal in the soil compost environment.

### *2.1. Laboratory soil reactors*

Laboratory soil compost reactors were constructed from 125 ml Erlenmeyer flasks equipped with a purge and trap gas sampling system (Fig. 2). Volatile organic material was captured in glass impingers containing ethylene glycol monomethyl ether while carbon dioxide was trapped in impingers containing monoethanolamine, methanol, and Ready Gel™ scintillation cocktail in a 1:4:5 (volume) ratio.

The reactor head space was purged with humified oxygen gas once every three days for 5 min to ensure aerobic conditions during soil treatment. Visual observation of white fungal growth on both soil and corn cobs during incubation confirmed aerobic conditions within the reactor. The quantity of radioactivity associated with volatile organic compounds and carbon dioxide was determined by measuring the radioactivity in impinger solutions by liquid scintillation (Beckman LS Model 1701).

### 2.2. Soil characteristics

An uncontaminated silt loam soil (Kidman series) obtained from Utah State University Experiment station in Kaysville, Utah was used as the test bench mark soil throughout the experimental program. Soil characteristics are given in Table 1.

### 2.3. Experimental design

Ten grams of air dried Kidman soil were added to each compost reactor. Each compost reactor received a chemical spike of methylene chloride (0.1 ml) containing nonradiolabeled and radiolabeled benzo(*a*)pyrene that resulted in a PAH and radioactivity soil concentration of 150 μg/g-soil and 0.03 μCi/g-soil, respectively. Distilled deionized water (DDW) was then added to bring the moisture content to 50% of field capacity.

Since benzo(*a*)pyrene cannot serve as a substrate for microbial growth, a suitable carbon and energy source was required. Corn cobs were selected as primary growth substrate since they: (1) were known to be utilized by *Phanerochaete chrysosporium* as an energy source, (2) act as an effective compost bulking agent and (3) are inexpensive. Five grams (dry weight) of fungus inoculated (or fungus uninoculated) corn cobs previously saturated with distilled deionized water were added to each microcosm and mixed with ten grams of contaminated Kidman soil.

Fungal inoculum was prepared by adding a fungal conidia suspension to the moistened corn cobs. The inoculated corn cobs were incubated at 39 °C under aerated conditions. White rot fungus, *Phanerochaete chrysosporium* BKMF-1767, was obtained from the Utah State University Biotechnology Center. The inoculum was maintained at 39 °C on 4% malt agar slants in a 250 ml Wheaten bottle with an

Table 1
Characteristics of Kidman Sandy Loam Soil[a]

| Characteristic | Value |
|---|---|
| *Physical properties* | |
| Bulk density | 1.5 g/cm$^3$ |
| Texture | Sandy loam |
| Moisture at 1/3 bar | 20% |
| Soil classification: | Typic haplustoll |
| *Chemical properties* | |
| pH | 7.2 |
| CEC | 10.1 meq/100 g |
| Organic carbon | 0.5% |
| *Biological properties* | |
| Soil plate counts | |
| Bacteria | $6.7 \times 10^6$ CFU/g |
| Fungi | $1.9 \times 10^4$ CFU/g |

[a] Analyzed by the soil, plant and analysis laboratory at Utah state university.

aerobic head space and transferred to fresh medium once every month. Conidia taken from a four week old slant were shaken with 100 ml distilled deionized water. The fungal suspension was used to inoculate 2 kg of dry corn cobs.

For each experimental condition, nine sets of compost reactors were monitored. Each set consisted of triplicate fungal amended reactors, triplicate unamended reactors, duplicate poisoned reactors and duplicate controls (no benzo(*a*)pyrene addition). A set of reactors was analyzed after 1, 7, 14, 21, 28, 35, 84, 91, and 95 days of incubation. At the selected time intervals, soil reactors were chemically extracted for benzo(*a*)pyrene, $^{14}C$, and chemical intermediates using the Soxhlet extraction procedure (SW-846, Method 3540 US EPA). The extraction solvent solution consisted of 200 ml of a 1:1 mixture of methylene chloride and acetone (volume basis). After evaporation of the organic solvent, the extracted residue was dissolved in acetonitrile for benzo(*a*)pyrene analysis using high performance liquid chromatography (HPLC) (SW-846, Method 8310 US EPA).

Benzo(*a*)pyrene was quantified on a reverse phase HPLC (Perkin Elmer Series 4 LC-90) system equipped with a 250 m × 2.6 mm I.D. stainless steel column filled with HC-ODS Sil-X (Supelco, Inc. Bellefonte, Pa). The HPLC was operated at a flow rate of 1.7 ml/min with 100% acetonitrile solvent for 8 min. Absorbance was measured at 254 nm.

### *2.4. Poisoned treatment*

Poisoning of soil reactors was accomplished by adding 3 ml of a 4% mercuric chloride to selected reactors. Past experience has shown that this concentration was effective in significantly inhibiting microbial growth.

### *2.5. Bound residue formation*

Bound residue formation was estimated from soil samples after organic solvent extraction. The procedure consisted of subsampling and combusting 1 g of solvent extracted soil using the Harvey Biological Oxidizer (Model OX400). The $^{14}CO_2$ evolved was determined by liquid scintillation (Beckman LS Model 1701).

### *2.6. Quality assurance and quality control*

Analytical procedures for analysis were conducted using standard methods from "Test Method for Evaluating Sold Waste, Physical/Chemical Methods" [16]. Benzo(*a*)pyrene recovery efficiencies were measured on triplicate samples. Poisoned and blank controls were monitored routinely to evaluate abiotic transformations and instrument response, respectively.

## 3. Results

Quality control procedures indicated that the recovery efficiencies of benzo(*a*)pyrene from soil compost systems were 101.9 ± 3.0% measured on triplicate

samples. Moreover, no benzo(*a*)pyrene was detected in any of the blank control reactors. Quality assurance and quality control data indicated that both the extraction procedures and instrument response were within acceptable levels according to EPA methods.

### 3.1. *Verification of Phanerochaete chrysosporium in soil reactor system*

Using a Martin rose bengal media and Cal Zeis contrast microscope, *P. chrysosporium* was observed at concentrations of greater than $1 \times 10^6$ CFU/gm soil in fungal inoculated systems. In compost systems receiving corn cobs only, the concentration of *P. chrysosporium* were approximately $1 \times 10^4$ CFU/gm after 95 days of soil treatment. In poisoned compost systems, no *P. chrysosporium* was detected which confirmed the effectiveness of the microbial poison.

### 3.2. *Benzo(a)pyrene transformation*

The change of benzo(*a*)pyrene concentration during soil compost treatment under the three test conditions is given in Table 2. The concentration data in Table 2 are depicted graphically in Fig. 3. The average contaminant removal efficiency in the poisoned control reactors over the 95 day treatment period was 49.3 ± 0.5% indicating that soil chemistry was effective in transforming B(*a*)P. It should be noted that abiotic transformation of B(*a*)P was nearly complete after 14 days of treatment. The extent of contaminant removal in abiotic controls after 14 days was 41.9% compared to 49.3% observed after 95 days of treatment. The fungal amended and unamended systems had removal efficiencies of 62.8 ± 5.9% and 65.6 ± 1.2%, respectively over the 95 day incubation period. Over the initial 14 days of incubation, all three systems

Table 2
Benzo(*a*)pyrene removal during soil composting process

| Incubation time (days) | Poisoned control | | Uninoculated | | Fungal inoculated | |
|---|---|---|---|---|---|---|
| | Ave. (%) | Stdev. (%) | Ave. (%) | Stdev. (%) | Ave. (%) | Stdev. (%) |
| 1 | 13.6 | 3.2 | 21.9 | 0.1 | 32.5 | 0.5 |
| 7 | 34.6 | 1.6 | 32.3 | 1.9 | 44.1 | 13.0 |
| 14 | 41.9 | 1.1 | 42.3 | 4.5 | 44.6 | 0.5 |
| 21 | 43.0 | 2.7 | 49.3 | 4.6 | 51.6 | 6.2 |
| 28 | 42.7 | 0.9 | 43.5 | 0.7 | 60.7 | 3.5 |
| 35 | 47.2 | 3.0 | 44.7 | 3.5 | 49.5 | 0.8 |
| 84 | 46.8 | 0.1 | 61.8 | 18.1 | 60.8 | 8.4 |
| 91 | 46.7 | 0.6 | 74.3 | 9.1 | 58.9 | 3.5 |
| 95 | 49.3 | 1.5 | 65.6 | 1.2 | 62.8 | 5.9 |

Ave.: average concentration (percent of initial value).
Stdev.: standard deviation.

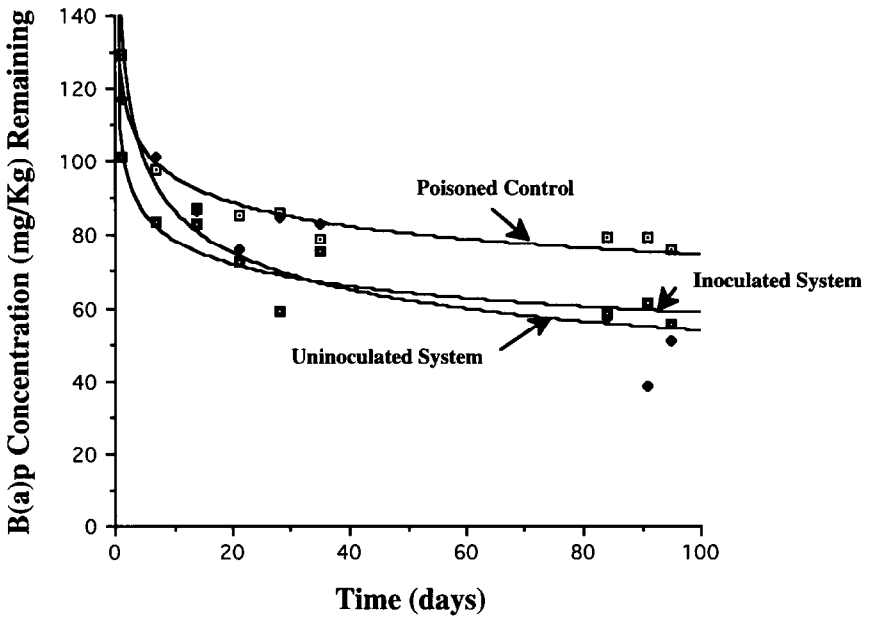


Fig. 3. Residual benzo(*a*)pyrene concentration in compost reactor.

demonstrated similar rates and extent of B(*a*)P removal. This suggests that not only is chemical removal predominant in the initial stages of treatment but that microbial acclimation is necessary for enhanced removal.

Statistically, the difference in removal effectiveness between the poisoned control compost reactors and the other systems was significant. However, the difference in removal efficiencies between fungal inoculated and uninoculated compost systems was insignificant. This suggests that bioaugmentation of soil compost systems with *Phanerochaete chrysosporium* may not be effective in enhancing the removal of benzo(*a*)pyrene over systems amended with organic material only.

Modeling benzo(*a*)pyrene removal using first order kinetics yielded decay coefficients of 0.08, 0.06 and 0.003 $d^{-1}$ which corresponded to chemical half lives of 11.5, 8.6 and 230 days for the fungal unamended, fungal amended and poisoned control compost reactors, respectively.

During poisoned and fungal amended test conditions, benzo(*a*)pyrene was observed to be removed rapidly over the first few days of treatment followed by a declining removal rate that approached zero by the end of the 95 day incubation period. This suggests the possibility of irreversible adsorption (or partitioning) of B(*a*)P to compost materials. The unamended system also demonstrated a rapid initial rate of benzo(*a*)pyrene removal followed by a declining rate. However, removal of B(*a*)P continued in the unamended fungal compost reactor throughout the 95 day incubation period.

### 3.3. Volatile intermediates

Analysis of all impingers for radioactivity indicated that less than 0.03% of the applied radiolabel could be accounted for in solvent traps. This suggests that not only is volatilization of benzo(*a*)pyrene or its chemical intermediates insignificant but that mineralization is not a major removal mechanism within the soil compost environment.

### 3.4. Bound residue formation

The extent of bound residue formation was estimated by measuring the radioactivity remaining in soil compost samples after solvent extraction (Table 3). Both the poisoned and fungal amended systems were characterized by a declining rate of bound residue formation. The unamended system maintained a constant bound residue formation rate of 0.64 mg B(*a*)p/kg material-day over the 95 day test period.

Although the addition of fungal inoculum resulted in a declining rate of bound residue formation over the 95 day incubation period, analysis of the short term (i.e., 30 day) effects of bioaugmentation suggested that fungal inoculation increased the initial rate of bound residue formation. The fungal inoculated system had an average rate of bound residue formation of 1.58 mg B(*a*)p/kg-day compared to 0.73 mg B(*a*)p/kg-day for the uninoculated system over the first thirty days of incubation.

Comparisons of benzo(*a*)pyrene removal efficiencies with the extent of bound residue formation indicates that approximately 100% of the contaminant removal could be accounted for as bound residue in the nonpoisoned systems. In the poisoned system, only 60% of the benzo(*a*)pyrene removal was accountable in the bound residue. The remaining 40% was found in chemical intermediates collected in the

Table 3
Bound residue formation during soil composting process

| Incubation time (days) | Poisoned control | | Uninoculated | | Fungal inoculated | |
|---|---|---|---|---|---|---|
| | Ave. (%) | Stdev. (%) | Ave. (%) | Stdev. (%) | Ave. (%) | Stdev. (%) |
| 1 | 0.96 | 0.07 | 2.05 | 0.76 | 3.76 | 0.28 |
| 7 | 6.08 | 1.29 | 6.07 | 0.86 | 13.37 | 9.27 |
| 14 | 14.58 | 0.29 | 12.56 | 0.69 | 15.67 | 2.51 |
| 21 | 13.45 | 2.00 | 13.59 | 1.91 | 20.05 | 9.09 |
| 28 | 24.10 | 1.30 | 17.20 | 1.54 | 36.69 | 3.69 |
| 35 | 31.48 | 5.36 | 25.14 | 5.40 | 24.24 | 1.17 |
| 84 | 25.19 | 2.25 | 38.97 | 16.73 | 30.33 | 5.61 |
| 91 | 17.76 | 11.12 | 50.72 | 9.54 | 35.51 | 5.30 |
| 95 | 24.58 | 1.92 | 40.42 | 4.00 | 37.58 | 8.22 |

Ave.: average concentration (percent of initial $^{14}C$ supplied).
Stdev.: standard deviation.

organic solvent extract. Although no attempt was made to identify these chemical intermediates, previous studies have indicated that B(*a*)P quinones and B(*a*)P hydroxyacids are the major chemical intermediates [17, 18].

A chemical mass balance approach was performed by following the radiolabel from the contaminant carbon during soil compost treatment. The sum of radioactivity found in solvent extracts, bound residue and volatilization traps was compared to the total radioactivity initially added to each reactor. An average $^{14}C$ percent recovery of $103.13 \pm 7.0$, $96.0 \pm 14.1$, and $96.5 \pm 7.2$ was determined at each measurement event for the poisoned, fungal unamended and fungal amended compost systems, respectively.

## 4. Discussion

The present study indicates that bioaugmentation of the soil compost system with the white rot fungus *Phanerochaete chrysosporium* was ineffective in enhancing the removal of benzo(*a*)pyrene during the 95 day soil treatment period over that observed in the system receiving organic nutrients alone. This result was not surprising given the fact that both the fungal amended and unamended compost systems were found to have substantial concentrations of *P. chrysosporium* ($> 1 \times 10^4$ CFU/gm) at the end of the treatment period. Since care was taken not to cross contaminate corn cobs, these data suggest that amending soils with known fungal substrates will suffice in encouraging growth of *P. chrysosporium* populations already present in soil. Moreover, results from the present kinetic study suggest that over long incubation periods (i.e., greater than 30 days), indigenous microbial species are as effective in degrading benzo(*a*)pyrene in the soil compost systems when supplied with appropriate organic nutrients as systems which receive nutrients plus microbial inoculum.

The decline in benzo(*a*)pyrene removal and bound residue formation rates in the fungal amended system suggests the possibility of nutrient limitations. In other words, bioaugmentation may result in enhanced rates of nutrient (i.e., carbon) uptake during cometabolic degradation of benzo(*a*)pyrene. This enhanced removal of organic nutrients reduces microbial activity during long term soil treatment. This may be an important engineering design consideration for fungal amended compost treatment since it has been reported that under carbon limiting conditions, the peroxidase activity from *P. chrysosporium* is severely inhibited [19].

Results from the present study indicate that the chemical nature of the organic matter may be critical in stimulating indigenous biotransformation reactions. Like other environmental parameters, the influence of the organic amendment in selecting for specific types and numbers of indigenous microorganisms must be considered for effective soil remediation.

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